Center for Veterinary Biologics
and
National Veterinary Services Laboratories
Testing Protocol

Supplemental Assay Method to Test for Bactericidal Activity of Diluents Used with Live Bacterial Vaccines

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Contact Person: Gerald G. Christianson, (515) 663-7416

Approvals:

_____ Date:_____
Gerald G. Christianson, Head
Sterility/Extraneous Agents Section

_____ Date:_____
Ann L. Wiegers, Quality Assurance Manager

_/s/ Randall L. Levings_____ Date:_11/5/99_
Randall L. Levings, Director
Center for Veterinary Biologics-Laboratory

United States Department of Agriculture
Animal and Plant Health Inspection Service
P. O. Box 844
Ames, IA 50010

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1. Introduction

1.1 Background

This Supplemental Assay Method (SAM) is used in the detection of bactericidal activity of bacterins used as diluents in the rehydration of live bacterial vaccines. Bacterins are compared with sterile purified water. This procedure may also be used to test for bactericidal activity of diluents used in the rehydration of live bacterial vaccines.

1.2 Keywords

Bactericidal activity, live bacterial vaccines, potency test, bacterin, diluent

2. Materials

2.1 Equipment/instrumentation

2.1.1 Sterile disposable syringes with needles

2.1.2 35° $\pm$ 2°C incubator

2.1.3 Disposable petri dishes

2.1.4 Sterile disposable cotton-plugged pipettes

2.1.5 Propipette or pipette bulb

2.1.6 Screw-capped tubes

2.1.7 Spreader

2.1.8 Biosafety cabinet

2.1.9 Magnetic stirrer

2.2 Reagents/supplies

2.2.1 Media: Specified in the firm's Outline of Production (OP) or the most current version of the SAM or Testing Protocol (PRO) for the live bacterial vaccine being tested.

3. Preparation for the test

3.1 Personnel qualifications/training

The personnel performing the test must have experience or training in this SAM. This includes knowledge of aseptic biological laboratory techniques and preparation, proper handling, and disposal of biological agents, reagents, tissue culture samples, and chemicals. The personnel must also have knowledge of safe operating procedures and policies and Quality Assurance (QA) guidelines of the Center for Veterinary Biologics-Laboratory (CVB-L) or equivalent, as well as training in the operation of the necessary laboratory equipment listed in **Section 2.1**.

3.2 Preparation of equipment/instrumentation

3.2.1 Turn on biosafety cabinets at least 1 hr before testing is started.

3.2.2 Monitor the temperature of incubators daily, according to the current version of GDOCSOP0001.

3.2.3 Monitor freezers and coolers daily for temperature according to the current version of GDOCSOP0003.

3.3 Preparation of reagents/control procedures

3.3.1 Technique Controls: Using the same disposable syringes or pipettes used with the test, inoculate 3 agar plates (specified in the OP) with 0.1 ml of the purified water used as diluent with each test conducted.

3.3.2 Negative or Media Controls: Incubate 3 uninoculated plates of each agar type utilized in the testing and 3 tubes of the dilution media to confirm the sterility of the agar plates and dilution media.

3.4 Preparation of the samples

3.4.1 Receive the biologics samples to be tested from the Biological Materials Processing Section according to the current version of STSOP0001.

3.4.2 Check the serial numbers of all biologics samples and diluents and record on the test sheets.

3.4.3 Order sufficient media of the types specified in the firm's OP, or in the appropriate SAM or PRO, from the NVSL media preparation department. Store plates used for making counts at refrigerator temperature. Plates to be used for counts are either placed in a 35° ± 2°C incubator overnight or dried in a biosafety cabinet prior to use. Plates must be no more than 14 days old.

3.4.4 Order sufficient volumes of sterile purified water, if required, in serum vials, from the NVSL media prep department, in sufficient volumes, as stated on the label or in the firm's OP.

4. Performance of the test

4.1 Disinfect the tops of all vials with 70% alcohol and flame before rehydration. Reconstitute 2 vials of vaccine with 2 vials of bacterin or diluent to be used as the rehydrant. Rehydrate the vaccine with the amount of bacterin or diluent specified on the vaccine label, using a plastic syringe of appropriate size. Mix the samples of vaccine thoroughly by inverting and shaking until the sample is completely dissolved.

4.2 Rehydrate 2 additional vials of the same serial of vaccine with sterile purified water.

4.3 Allow the 4 vials of rehydrated vaccine to sit at room temperature (20°-25°C) for 2 hr.

4.4 Dispense 4.5 ml of the appropriate diluent (listed in the firm's OP or appropriate SAM or PRO) into sterile screw-capped tubes. These will serve as blanks for the serial tenfold dilutions.

4.5 Transfer 0.5 ml of the vaccine sample from each of the 4 vials to 4 screw-capped tubes containing 4.5 ml of diluent. These tubes are the 1:10 (10^{-1}) dilutions of the 4-dilution series. Make serial dilutions to 1 dilution above the recommended release titer as specified in the OP. Inoculate a set of 3 plates per dilution from the 3 highest consecutive dilutions.

4.6 Mark the plates with the sample number, dilution, and vial number. Inoculate each plate with 0.1 ml of the appropriate tenfold dilution, using a pipette. Use a sterile spreader to evenly distribute the inoculum over the surface of the agar. Invert the plates and incubate at the temperature and for the time period specified in the OP or appropriate SAM or PRO.

4.7 Count the colonies on the plates of the dilution that have between 30 and 300 colonies per plate.

4.8 Average the colony counts of the 2 vials rehydrated with bacterin or diluent and those 2 vials rehydrated with sterile water separately.

4.9 Calculate the colony-forming units (CFU) per dose for the 2 averages.

5. Interpretation of the test results

5.1 If the titer of the live bacterial vaccine rehydrated with the bacterin or diluent is more than 0.7 log 10 below the titer of vaccine rehydrated with sterile purified water, the bacterin is unsatisfactory for use as diluent for performing the potency test on the product.

5.2 If the bacterin or diluent is unsatisfactory in the first test, 1 retest to rule out faulty technique may be conducted using 4 vials rehydrated with bacterin or diluent and 4 vials rehydrated with sterile purified water. The acceptability of the bacterin or diluent for use when potency testing the product will then be judged by the results of the second test.

6. Report of test results

Record the results of this testing in the log book for the potency testing of live bacterial vaccines.

7. References

Code of Federal Regulations, Title 9, Part 113.25, U.S. Government Printing Office, Washington, DC, 1999.

8. Summary of revisions

This document was rewritten to meet the current NVSL/CVB QA requirements, to clarify practices currently in use in the CVB-L, and to provide additional detail. No significant changes were made from the previous protocol.